Technical note: Artificial vagina vs a vaginal collection vial for collecting semen from rams

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Technical note: Artificial vagina vs a vaginal collection vial for collecting semen from rams[1]

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ABSTRACT: The time required to train rams to an artificial vagina (AV) makes collecting semen from large numbers of rams difficult. To manage this problem, we developed a glass, round-bottomed, 1.9-cm i.d. × 9.8-cm long vaginal collection vial (VCV). Three experiments were conducted to determine whether the VCV affected 1) semen volume per collection, 2) percentage of motile spermatozoa, 3) forward progressive motility score before and after extension and after freezing and thawing, and 4) our ability to collect semen from untrained rams. A soft rubber cap with a hole in the center was used to cover the VCV. A VCV was inserted into the vagina of an estrual ewe, and a monofilament line attached to the VCV was clipped to the wool near the vulva. Rams were joined with unrestrained ewes in a pen until they ejaculated into the VCV. In Exp. 1, five rams trained to an AV were used in a switchback design with four collection periods. During each period (1 d), semen was collected with an AV and a VCV. Immediately after collection, semen volume and sperm motility were quantified. Semen was extended with an aloe vera gel-based diluent at a 1:4 dilution rate, motility was quantified again, and semen was frozen. At 1 h after freezing, semen was thawed and sperm motility was quantified. Ejaculate volume (mean = 0.7 mL) and all measures of motility after collection were similar ($P > 0.05$) for the two collection methods. In Exp. 2, 10 rams trained to an AV were used in a switchback design with five collection periods (period = 3 d). On d 1 and 3 of each period, an AV and a VCV were used to collect semen. Collection method did not affect ($P > 0.05$) ejaculate volume (mean = 1.0 mL), percentage of motile cells, or forward progressive motility score. In Exp. 3, 51 untrained rams were used in a switchback design with a single collection period (2 d). Semen was collected with an AV and a VCV. Ability to collect an ejaculate and time required for collection were recorded. The likelihood of collecting semen from untrained rams was greater ($P < 0.01$) using a VCV (mean = 31.4%) than using an AV (mean = 9.8%). Collection method did not affect ($P > 0.05$) ejaculate volume (mean = 0.8 mL), percentage of motile cells, or forward progressive motility score. We concluded that a VCV could be used to collect semen from rams that are not trained for semen collection without decreasing ejaculate volume or sperm motility.

Key Words: Artificial Insemination, Artificial Vagina, Rams, Semen

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Introduction

Artificial insemination can increase the rate of genetic improvement. However, several factors limit the use of AI in sheep, including difficulties associated with collecting semen from large numbers of untrained rams. Typically, ram semen is collected in an artificial vagina (**AV**) or by electrical stimulation. Collection with an AV resembles natural service but usually requires a preliminary training period. The training period can be brief or as long as 3 wk, depending on the individual rams (Terrill, 1940). Electroejaculation is faster and more convenient than using an AV, but semen quality is often diminished (Brady and Gildow, 1939; Terrill, 1940; Mattner and Voglmayr, 1962). This study was conducted to develop and determine the efficacy of a vaginal collection vial (**VCV**), which is a new type of collection device designed to overcome problems associated with collecting semen from large numbers of untrained rams.

Materials and Methods

The USDA, U.S. Sheep Experiment Station Animal Care and Use Committee approved all animal protocols.

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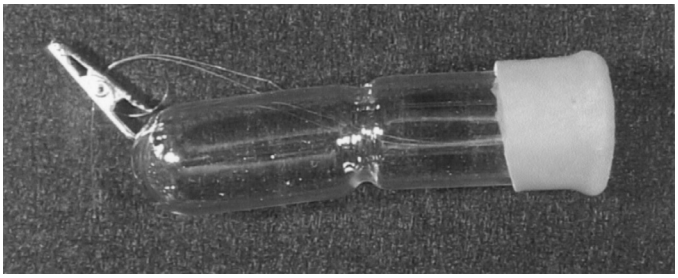

Figure 1. Vaginal collection vial.

Vaginal Collection Vial and Its Development

A series of vaginal inserts was evaluated using 51 rams and eight ewes to determine whether use of a vaginal insert would be an appropriate method of semen collection. Initially, two sizes (10 and 15 cm) of Whirl-Pak (Nasco, Fort Atkinson, WI) plastic bags were used. Neither size of Whirl-Pak was suitable. Rams were able to ejaculate in the vagina outside of the Whirl-Pak, or the Whirl-Pak was torn. In an attempt to reduce flexibility and increase rigidity, latex collection cones were used, but they were not noticeably better than the Whirl-Paks.

Thus, a stronger and more rigid collection vial was needed. Glass vials resembling test tubes with various internal diameters and lengths were evaluated. Three sizes of glass vials were evaluated: small (1.75 cm i.d. × 9 cm long), medium (1.9 cm i.d. × 9.5 cm long), and large (2.2 cm i.d. × 9.8 cm long). The sizes were based on the internal diameter of a ewe's vaginal canal and the external diameter of a ram's penis. Even though the small vial was the easiest to manipulate into a ewe's vagina, it was unacceptable. It left too much space on either side of the vial and allowed a ram to insert his penis into the vagina without inserting it into the vial. The internal diameter of the small vial was also too small for the penis of many of the rams. The large vial better accommodated the rams; however, it was often too large to easily manipulate into a ewe's vaginal canal. Therefore, the medium-sized vial was selected.

In its initial design, the VCV was a straight glass tube; however, it was difficult to keep a straight glass tube in the vagina. Therefore, midway down its length, the VCV was bent at a 10° angle. This bend helped secure the VCV in the vagina.

Even though glass provided a rigid surface, the vial did not provide adequate pressure to cause ejaculation. Thus, a soft rubber cap with a hole in the center was used to cover the VCV. The soft rubber cap probably increased the pressure exerted on the penis, causing ejaculation.

Approximately 50% of the time, rams pulled the VCV out of the vagina when they dismounted a ewe. So, a monofilament line was attached to the VCV, and an alligator clip was attached to the free end of the line and used to clip the line to the wool near the vulva. The VCV prototype used for the study is shown in Figure 1.

General

Estrus Synchronization. Pessaries containing 60 mg of 6α-methyl-17α-hydroxyprogesterone acetate (Tuco Products Limited, Orangeville, Ontario, Canada) were inserted into ovariectomized ewes. Pessaries were removed after 12 d. Beginning at the time of pessary removal, 50 μg of estradiol-17β in sesame oil diluent (100 μg/mL) was injected i.m. daily to maintain estrus (modification of Lewis and Goebel, 1993). Ewes standing firmly to be mounted 48 h after pessary removal were considered to be in estrus and were used as stimulation and mount animals for semen collection.

Semen Collection. Rams were penned with two estrual ewes. For collecting semen with an AV, ewes were restrained in stanchions in the presence of a handler with an AV, as described previously (Frank, 1950). For collection with a VCV, ewes were unrestrained in a pen without a handler. A VCV was inserted into the vagina of each ewe before a ram was moved into the pen. To insert the VCV, the perineal area was scrubbed with an antiseptic soap, and excess water was removed with gauze sponges. The outside surface of a VCV was coated with nonspermicidal obstetrical lubricant. The VCV was inserted into the vaginal canal until only the monofilament line remained outside of the vulva. The line was then clipped to the wool. Rams were allowed 10 min of exposure time to mount and ejaculate.

Semen Evaluation. Semen was collected and transferred to graduated tubes, and the volume was determined to the nearest 0.1 mL. The percentage of motile spermatozoa was estimated to the nearest 10%. After estimating sperm motility, movement of motile spermatozoa was scored using a modification of Terrill's (1937) system. Briefly, forward progressive motility was scored from 4 to 1, with a score of 4 denoting the greatest forward progressive movement and a score of 1 denoting no motility.

Statistical Analysis

The GLM procedures of SAS (SAS Inst. Inc., Cary, NC) were used to determine the effects of collection method on ejaculate volume, motility measurements, and collection rate. The GLM procedures using least squares methods have advantages over chi-squared methods for analyzing the same binomial data (Ercanbrack and Knight, 1998). Li (1964) concluded that either chi-squared or analysis of variance with least squares methods can be used for testing hypotheses that means are equal and that the conclusions are usually the same.

Experiment 1

Five rams trained to an AV were used in a switchback design with four collection periods. During each period (1 d), semen was collected with an AV (total AV collections = 20) and a VCV (total VCV collections = 20). Immediately after collection, semen volume and motil-

ity were quantified. Semen was extended with an aloe vera gel-based diluent at a 1:4 dilution rate, motility was quantified again, and semen was frozen (Rodriguez, 1991). At 1 h after freezing, semen was thawed and motility was quantified.

Experiment 2

Ten rams trained to an AV were used in a switchback design with five collection periods (period = 3 d). On d 1 and 3 of each period, either an AV (total AV collections = 50) or a VCV (total VCV collections = 50) was used to collect semen. Immediately after collection, ejaculate volume and sperm motility were quantified.

Experiment 3

Fifty-one untrained rams were used in a switchback design with a single collection period (2 d). Semen was collected with an AV (total AV collection sessions = 50) and a VCV (total VCV collection sessions = 50). Ability to collect an ejaculate and time required for collection were recorded. Immediately after collection, ejaculate volume and sperm motility were quantified.

Results

Experiment 1

Period was not significant and was dropped from the model for further analysis. Ejaculate volume and motility at all times (i.e., before extension, after extension, or after freezing and thawing; Table 1) were similar between the two collection methods.

Experiment 2

Period was not significant and was dropped from the model. Collection method did not affect ($P > 0.05$) ejaculate volume, percentage of motile cells, or forward progressive motility score (Table 2).

Experiment 3

We were more likely ($P < 0.01$) to collect semen from untrained rams using a VCV (mean = 31.4%) than from untrained rams using an AV (mean = 9.8%). Collection method did not affect ($P > 0.05$) ejaculate volume, percentage of motile cells, or forward progressive motility score (Table 3).

Discussion

The time required to train rams to an AV makes collecting semen from large numbers of rams difficult. In addition to the time requirement, training rams and collecting semen with an AV can be hazardous to handlers. Therefore, we developed a method for collecting semen that does not require training the rams or the presence of a handler in the collection pen.

Table 1. Semen characteristics after collection with either an artificial vagina (AV) or a vaginal collection vial (VCV) in Exp. 1

	Treatment[a]		
Variable	AV	VCV	SEM
Ejaculate volume, mL	0.7	0.6	0.08
	Immediately after collection[b]		
Sperm motility, %	62.5	61.8	1.10
Motility score[e]	3.0[f]	3.0[f]	
	Diluted[c]		
Sperm motility, %	64.6	62.9	0.89
Motility score[e]	3.0[f]	3.0[f]	
	Post-thawd		
Sperm motility, %	43.3	44.3	1.18
Motility score[e]	3.0	2.9	0.05

[a]Five rams trained to an AV were used in a switchback design with four collection periods. During each period (1 d), semen was collected with an AV and a VCV. Row values do not differ ($P > 0.05$).
[b]Immediately after collection, semen volume and sperm motility were quantified.
[c]Semen was extended with an aloe vera-based diluent at a 1:4 dilution rate, motility was quantified again, and semen was frozen.
[d]One hour after freezing, semen was thawed and sperm motility was quantified.
[e]Motility score: 4 = high degree of forward progressive motility to 1 = no motility.
[f]Motility score was the same for all samples, and SEM = 0.

Based on the ability to obtain an ejaculate within a 10-min period, collection of semen from untrained rams with a VCV was more effective than collection of semen with an AV. More specifically, we were able to collect semen from approximately 31% of the untrained rams using a VCV and from only approximately 10% of the untrained rams using an AV. Ideally, we would like to obtain collections from 100% of rams, which was the case in Exp. 1 and 2 with rams that had already been trained to an AV. This seems to indicate that our ability to collect semen increases as rams become familiar with the location and activities associated with semen collection. Because collection with a VCV is less intrusive than collection with an AV, it may be possible to increase our collection rate by simply familiarizing rams

Table 2. Semen characteristics after collection with either an artificial vagina (AV) or a vaginal collection vial (VCV) in Exp. 2

	Treatment[a]		
Variable	AV	VCV	SEM
Ejaculate volume, mL	1.0	1.1	0.05
Sperm motility, %	42.4	43.6	1.01
Motility score[b]	2.6	2.5	0.06

[a]Ten rams trained to an AV were used in a switchback design with five collection periods (period = 3 d). On d 1 and 3 of each period, either an AV or a VCV was used to collect semen. Row values do not differ ($P > 0.05$).
[b]Motility score: 4 = high degree of forward progressive motility to 1 = no motility.

Table 3. Collection rate, collection time, and semen characteristics after collection with either an artificial vagina (AV) or a vaginal collection vial (VCV) in Exp. 3

	Treatment[a]		
Variable	AV	VCV	SEM
Collection rate, %[b]	9.8[e]	31.4[f]	0.07
Collection time, s[c]	224	230	37.6
Ejaculate volume, mL	0.6	0.9	0.12
Sperm motility, %	54.0	50.6	1.77
Motility score[d]	2.8	2.4	0.11

[a]Fifty-one untrained rams were used in a switchback design with a single collection period (2 d). Semen was collected with an AV and a VCV. Row values without superscripts do not differ ($P > 0.05$).
[b]Collection rate: (number of rams providing collections ÷ number of collection sessions for the treatment group) × 100.
[c]Rams were allowed 10 min to mount and ejaculate. If semen was collected in less than 10 min, collection time was recorded.
[d]Motility score: 4 = high degree of forward progressive motility to 1 = no motility.
[e,f]Means with different superscripts within the same row differ ($P < 0.01$).

with their surroundings and handlers before rams are joined with ewes for semen collection.

Ejaculate volume and motility estimates did not differ between the two collection methods in any of the three experiments. Semen collection with an AV has yielded the highest-quality samples of any collection method currently available (Terrill, 1940; Mattner and Voglmayr, 1962; Marshall and Hafs, 1972). Therefore, recovering samples with a VCV that are comparable to those recovered with an AV indicates that we can use a VCV without reducing semen quality.

To use AI to transfer superior genetics across flocks in the United States, a readily transportable source of semen is necessary. Indeed, the collection method must yield spermatozoa that can be frozen and thawed and still retain the ability to fertilize ova. Collection method did not affect post-thaw semen quality (ability to fertilize ova was not tested), indicating that semen collected with a VCV seems just as useful as semen collected with an AV.

In conclusion, our results with the VCV used in these three experiments are comparable to those with an AV, except that semen collection from untrained rams was more likely with a VCV than with an AV.

Implications

Based on descriptions of other methods and our experience, we believe that our semen collection method with a vaginal semen collection vial is simpler and safer than the others. Therefore, we concluded from these experiments that our vaginal semen collection vial could be used more effectively than an artificial vagina to collect semen from large numbers of untrained rams.

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